

ase, sometimes even in a great degree: this is probably due to the aggregation of the protein particles in larger masses as a result of the action of the temperature: the action of the enzyme on the contrary seems to be of no importance in this respect, since an increase of the extinction values ranging within the same limits has been observed also after extraction with water.

The values of E_{265} - E_{315} appear to be markedly reduced after digestion by ribonuclease; the values of E_{280} - E_{315} too are decreased, in the same or in a slightly lesser degree than the former. Also after extraction with water there is a more or less considerable reduction of the extinction values, but it is in any way much lesser than after treatment with ribonuclease. This large variability in the effects of water extraction, which does not appear to be in a close relationship with the initial extinction values, makes it impossible to establish, in the case of digestion by ribonuclease, how much is due to the action of the enzyme and how much to the water extraction. On the other hand, it should be noted that, according to BRATTGÅRD and HYDÉN¹, the digestion by ribonuclease cannot be compared with a simple water extraction, since the enzyme has a denaturing effect on the protein structures; in fact, after treatment with ribonuclease, those authors did not observe any loss in weight, determined by X-ray microradiography, other than that which could be referred to the action of the enzyme. On the basis of this assumption, the marked reduction of the extinction values at 2650 Å, after digestion by the enzyme, is due to the remotion of PNA and, in a much lesser degree, to the release of the proteins, which are attached to the PNA and which go into solution together with the splitting off of it; the reduction of the extinction at 2800 Å, on the other hand, must be referred to the same factors, except that the release of the proteins bound to the PNA has, in such regard, a greater influence at this wavelength than at 2650 Å. A direct action of the ribonuclease on the proteins, on the contrary, should not be admitted, since the enzyme used in the present work had to be considered free from proteolytic activity.

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Résumé

L'auteur a fait une étude microspectrophotométrique des cellules de PURKINJE du rat blanc adulte. La courbe de distribution des fréquences montre une remarquable dispersion des valeurs d'extinction cytoplasmiques à 2650 Å et 2800 Å, avec un groupement des fréquences plus hautes vers les valeurs basses. L'étude des spectres d'absorption a démontré un maximum d'absorption des nucléotides et un maximum protéique, même dans le cytoplasme des cellules moins absorbantes.

La digestion des coupes par une ribonucléase dépourvue d'activité protéolytique a provoqué une diminution considérable des valeurs d'extinction cytoplasmique à 2650 Å et 2800 Å; aux mêmes longueurs d'onde l'ex-

traction par l'eau distillée a déterminé une réduction d'extinction très variable, mais en tout cas bien inférieure à celle provoquée par l'enzyme. Après la digestion par la ribonucléase, ainsi qu'après l'extraction par l'eau, on a observé une augmentation plus ou moins remarquable des pertes non spécifiques de lumière dues à la diffraction et à la réflexion dans la préparation.

A Model for the Cortical Reaction of Fertilization in the Sea-Urchin Egg

The disappearance of the cortical birefringence of the sea-urchin egg as a result of fertilization¹ has been tentatively interpreted as a disarrangement of the submicroscopic structure due to the splitting of the lipoprotein complex of the cortical layer through the action of some agent introduced by the sperm².

To check this hypothesis, a model experiment has been devised. As a model for the cortical layer of the unfertilized sea-urchin egg, the lipoprotein of the hen's egg-lipovitellin—has been prepared according to CHARGAFF³. Dialysis of the lipovitellin against sea-water results in a whitish emulsion. To this a fresh suspension of sperm of *Arbacia lixula* was added and at various intervals of time (from 2 to 15 min) free phospholipids were extracted with cold ether. The extract was evaporated, dried with anhydrous Na-sulphate and redissolved in ether and chloroform. After evaporation, lipid-P was determined. Zero time tubes were extracted with cold ether immediately upon addition of sperm to the lipovitellin. A certain amount of ether-extractable phospholipids was present in our preparations of lipovitellin. However, no further spontaneous release of phospholipids occurred on standing at room temperature for the time of the experiment. Therefore, together with each experiment a control was run in which sea-water was added to the lipovitellin instead of sperm.

μg of phospholipid-P released from lipovitellin under the influence of sperm of *Arbacia lixula*

Control (lipovitellin)	Time after mixing, in minutes				
	0	2	5	10	15
10.5	22.2	27.4	35.0	45.4	44.4
12.8	29.0				
22.4	32.4				
15.2	32.4				
9.8	12.2				15.1

The results of the experiments are summarized in the Table. They show that under the influence of sperm, phospholipids are released from the lipovitellin. This model—although certainly a very rough one—at least indicates that sperm are endowed with mechanisms which enable them to break-up lipoprotein bonds, thus releasing the phospholipids. That may be considered to support our interpretation of the cortical reaction of fertilization in the sea-urchin egg.

¹ A. MONROY, *Exper.* 1, 335 (1945). — A. MONROY and G. MONTALENTI, *Biol. Bull.* 92, 151 (1947). — LORD ROTHSCHILD and M. M. SWANN, *J. Exp. Biol.* 26, 164 (1949). — S. INOUÉ and K. DAN, *J. Morphol.* 89, 423 (1951).

² A. MONROY, *J. Cell. Comp. Physiol.* 30, 105 (1947).

³ E. CHARGAFF, *J. Biol. Chem.* 142, 491 (1942).

¹ S. O. BRATTGÅRD and H. HYDÉN, *Acta Radiol. Suppl.* 94 (1952).

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Riassunto

Da una soluzione di lipovitellina (la lipoproteina del tuorlo dell'uovo di pollo) trattata con spermi vivi di *Arbacia lixula* si ha liberazione di fosfolipidi. Questo esperimento è considerato un modello della reazione corticale di fecondazione dell'uovo di riccio di mare.

On the Incorporation of Radioactive Amino Acids into the Protein Fraction of *Torula utilis*

Torula utilis incorporates labeled carbon supplied in the form of DL-amino acids into its protein fractions by an enzymatic process¹. It is of interest to learn whether there is a preferential utilization of one optical isomer. When an inert isomer is added to the radioactive racemic mixture prior to incubation with the organism the isomer should decrease by isotope dilution the amount of radioactive protein produced if the isomer is identical with that member of the racemic mixture which the cells select for incorporation into the protein fraction. The results given in the table were obtained by incubating *Torula utilis* with DL-alanine-1-¹⁴C. Since there is a decrease in radioactivity of the protein fraction on addition of either the inert L or the D isomer, it must be assumed that the yeast cell utilizes both isomers for incorporation into the protein fraction.

Effect of Addition of Inert Optical Isomers on Incorporation of DL-alanine-1-¹⁴C into *Torula utilis* Proteins

Substance Added	Relative Radioactivity of Protein Fraction
Control, standard medium only	100
10 mg inert L-alanine	4.6
10 mg inert L-alanine	4.3
10 mg inert D-alanine	12.8
10 mg inert D-alanine	11.9

Each incubation flask contained 0.2 ml packed cells suspended in 2 ml standard medium (2) and 0.2 mg (2.4×10^{-3} mc) of DL-alanine-1-¹⁴C. The flasks were incubated at 37°C for 45 min in an oxygen atmosphere. The method of analysis has been described in a previous paper².

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Zusammenfassung

Der Eiweissanteil in Gegenwart von radioaktivem DL-Alanin gezüchteter Hefe zeigt eine Aktivitätsverminderung, wenn der Suspension nicht radioaktives D- oder L-Alanin zugefügt wird. Offenbar vermag die Hefezelle beide Isomere auszunützen.

¹ A. H. WEBB, F. FRIEDBERG, and L. M. MARSHALL, Biochim. biophys. Acta 6, 568 (1951).

² F. FRIEDBERG and A. H. WEBB, J. Bact. 58, 151 (1949).

L-Arginin als Bewegungsregulator des « Venenherzens »

Mit der völligen Isolierung einzelner Teilstücke von Flughautvenen der *Chiroptera* (Fledermäuse und Flughunde) und der Entwicklung des « Venensäckchen-Präparates »¹, haben wir die autochthone Pulsautomatie dieser glatt-muskulären Blutgefäße nachgewiesen. Dann konnte mit Binnendruckreizen der aktive Venenpuls ausgelöst und mit steigendem Druck die Pulsfrequenz erhöht werden. Die intravaskuläre Dehnung ist also ein natürlicher Reiz für die Venenperistaltik². Ferner ermittelten wir eine auffallende Temperaturabhängigkeit der Venenpulsfrequenz, welche über ihren gesamten biokinetischen Temperaturbereich von rund 45°C streng der van-t'Hoff-Arrhenius-Regel gehorcht³. Unsere Untersuchungen über die Beteiligung des Sauerstoffs bei der autonomen Venenpulsation⁴ wie auch die Temperaturversuche machen es wahrscheinlich, dass der Organisation der aktiven Pulsation ein vermutlich einfacher biochemischer Vorgang zugrunde liegt. Der Temperaturkoeffizient nach VAN T'HOFF Q_{10} entspricht jedenfalls dem, was man sonst von chemischen und enzymatisch gesteuerten Vorgängen her kennt.

Die Prüfung der elektrotonischen Erregbarkeitsänderungen⁵ und die Reaktionen der Vene auf weitere elektrische Reize⁶ ergaben Kriterien für die Primitivität des kontraktile Apparates. Auch beim Elektrogenogramm fanden wir ausgesprochen elementare Verhältnisse⁷.

Die Untersuchungen der ionalen Einflüsse auf diese Gefäße liessen erkennen, dass die Ionen selbst als Puls auslöser nicht in Frage kommen können. In der adäquaten Ringerlösung kommt die aktivpulsierende Vene relativ rasch zum diastolischen Stillstand⁸. Mit den bekannten kreislaufaktiven Wirkstoffen, wie Adrenalin, Histamin und Acetylcholin, wurden in physiologischen Konzentrationen nur geringfügige Effekte erzielt⁸.

Hingegen konnten wir mit dem dialysablen Anteil des Fledermausserums wie auch anderer Säugerseren meistens und ausgesprochen stark den Puls auslösen bzw. anregen und verstärken^{9,10}. Wir haben inzwischen auf der Suche nach diesem vom Blutplasma gelieferten humoralen Auslöser systematisch die freien Aminosäuren geprüft und im L-Arginin den spezifischen Reizstoff gefunden. Nach fermentativem Abbau des Arginins mit Arginase, nach Decarboxylierung oder papierchromatographischer Abtrennung des Arginins, unterbleibt die anregende Wirkung des zur Badeflüssigkeit (adäquate Ringerlösung) zugetropften Serums. Das L-Arginin (synthetisches und das aus dem Serum isolierte Arginin) bewirkt in Konzentrationen von 5 bis 100 γ pro cm³ an der isolierten Flughautvene regelmässig und langfristig drei typische Vorgänge: 1. Frequenzsteigerung, 2. Tonuserhöhung und 3. Amplitudenvertiefung. Die ausführliche Beweisführung für das L-Arginin als herzaktive Substanz erscheint zusammen mit unseren Befunden über die Wirkungsweise diverser Herzextrakte in « Helvetica physiologica Acta ». Wir halten hier nur das Hauptergebnis fest, dass der von uns früher als eiweissfreier Reizstoff im Serumdialysat eingeeengte myotrope Faktor des Venenherzens das L-Arginin ist.

¹ H. MISLIN, Helv. physiol. Acta 5, C3 (1947).

² H. MISLIN, Helv. physiol. Acta 5, C18 (1947).

³ H. MISLIN, Rev. suisse Zool. 48, 563 (1941).

⁴ H. MISLIN, Helv. physiol. Acta 7, C15 (1949).

⁵ H. MISLIN, Helv. physiol. Acta 6, C31 (1948).

⁶ H. MISLIN and M. KAUFMANN, Rev. suisse Zool. 56, 344 (1949).

⁷ H. MISLIN, Exper. 4, 28 (1948); Helv. physiol. Acta 8, C74 (1951).

⁸ H. MISLIN, Exper. 7, 385 (1951).

⁹ H. MISLIN, Z. f. Kreislaufforsch. 42, 620 (1953).